

Cryomapping on *Daphnia magna* at Beamline L using a cryostream: experimental setup and comparison.

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The analysis of metals within biological systems using X-ray fluorescence imaging is a quickly developing field of research. An inherent difficulty of micro-XRF imaging on biological materials is associated with the need to observe the sample in its natural state as much as possible. Sample structure should be preserved, and the elemental distributions within the sample should not change during analysis. However, most of the biological samples are containing high amounts of water, which slowly evaporates and changes the elemental distributions. Different sample preparation methods are currently available to remove the water content from biological samples such as critical point drying, freeze drying and dehydration through graded solutions followed by chemical fixation. However, removal of water contents can also cause an extra source of dislocation of elements and/or may introduce sample contamination with other metals.

An alternative method is to quickly freeze the sample (e.g. plungefreezing, high pressure freezing) so that all water is transformed to vitreous ice avoiding ice crystal formation, followed by the analysis of the sample in its native state at a temperature below the glass transition temperature of water (136K) so that no recrystallization occurs. In this report, we discuss the implementation of a cryostream cooler in the experimental set-up and make a comparison between the elemental distributions within a frozen, hydrated and a dehydrated, fixed *Daphnia magna* organism. *Daphnia magna* is a freshwater crustacean used in toxicological research as a model organism to evaluate the harmful effects of heavy metals on the ecosystem (ref.1).

Fig. 1 shows a photograph of the experimental set-up at beamline L. A 700 series cryostream cooler from Oxford Cryosystems (ref.2), normally used for synchrotron cryocrystallography, was installed under an angle of 45° with respect to the polarisation plane and the detector axis. A laminar gas flow of 5 L/min insured a temperature of 100 K at 8 mm distance from the cryostream nozzle. Icing can occur on objects outside the path of the laminar flow, therefore a single bounce capillary with a long working distance of 5 cm was used and the detector was shielded with a kapton foil to prevent damage to the Be window.

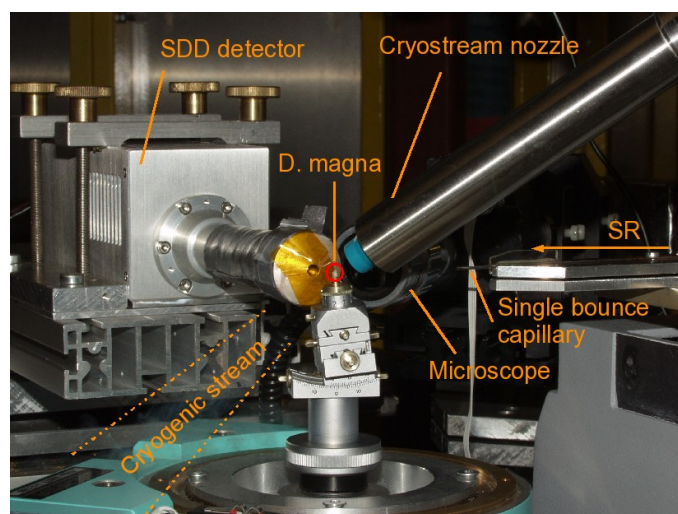


Fig. 1: Experimental set-up at Beamline L

Daphnia magna was collected from the culture medium, rinsed using deionised water and glued to a sample support fibre and inserted into the cryostream, which induced a fast freezing of the sample. Immediately afterwards, a 2D micro-XRF “cryomapping” was started with a 20 μm stepsize and 1.5 s scanning time/point resulting in a scanning time of approximately 6h. During this time, no icing on the sample was observed and vibrations of the sample due to the cryostream were estimated to be below the μm level based on microscope observation. A small bubble of frozen water could be observed outside the sample, probably due the transition of water into ice. At the same time, another sample was taken from the culture and underwent a HMDS drying procedure used in previous work (ref.3). Also, a 2D micro-XRF mapping was applied on this sample under identical experimental conditions (except the presence of the cryostream cooling).

Fig. 2 shows a comparison between elemental distributions of Zn, Cu and Br of a cryofrozen (left) and a chemically fixed (right) *Daphnia magna*. A colorbar has been added which shows the intensities in counts. It can immediately be observed that deformations of the tissue structure in the chemically fixed sample are present (e.g. more diffuse appearance of the tissue structures). However, the Zn concentration seems to be similar between both samples, with exception from upper gut and exoskeleton borders. Cu seems to show a significant leaching from the gut (also Fe and Mn show significant changes). The Br distribution appears to be very comparable (also Rb, Se, Sr are comparable). Another interesting observation is the large Compton scattering in the frozen sample due to its water content. The Compton map shows a uniform distribution, whereas in the dried sample the dried tissue structures are distinguishable based on the 2D Compton maps.

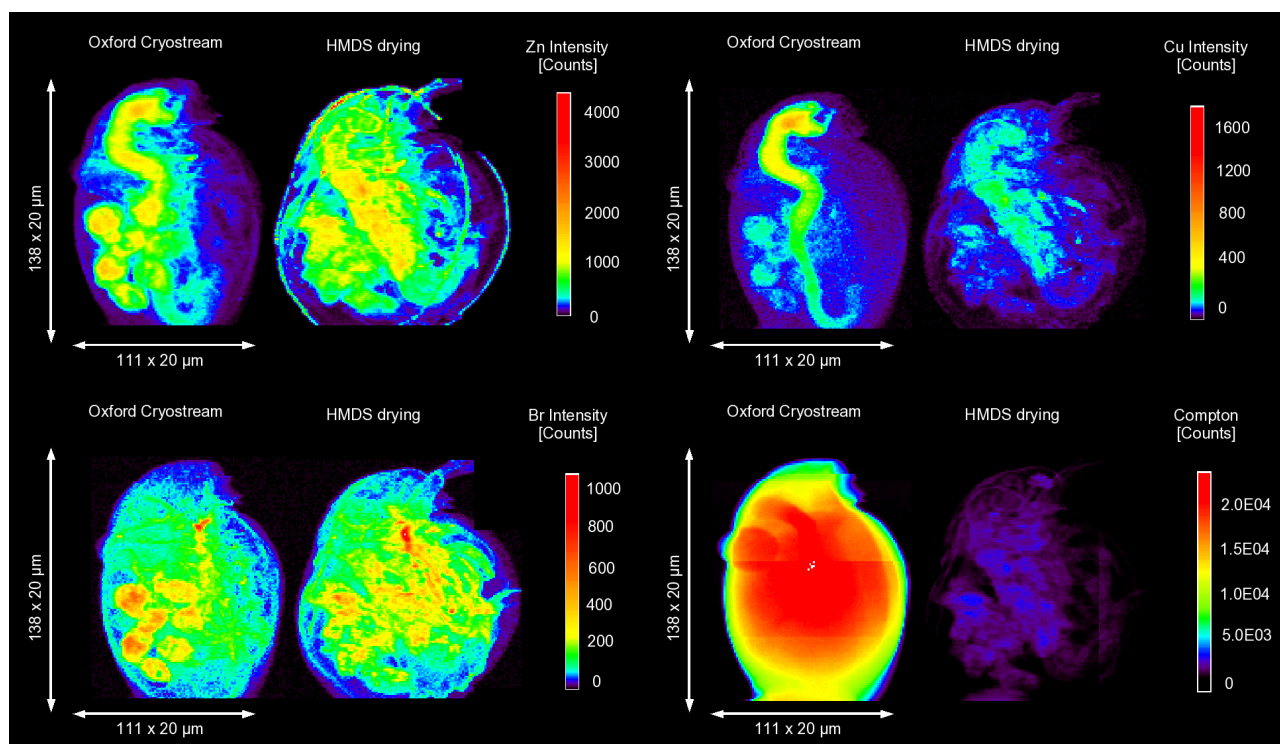


Fig. 2: comparison between the difference in Zn, Cu, Br and compton distribution between a chemically fixed and a cryofrozen *Daphnia magna*

It can be concluded that, when a cryostream is available, “cryomapping” is an excellent method to analyze the metal distributions within biological samples in their native state. Since icing problems were not observed on the sample and vibrations appear to be below the μm level, this method is fully compatible with the current Beamline L setup.

References:

- [1] Development of a chronic zinc biotic ligand model for *Daphnia magna*. Heijerick et al., *Ecotoxicology and Environmental Safety*, 2005, 62, 1-10.
- [2] OxfordCryosystems 700 series. (<http://www.oxfordcryosystems.co.uk/cryostream/700series.htm>)
- [3] A combination of synchrotron and laboratory X-ray techniques for studying tissue-specific trace level metal distributions in *Daphnia magna*. De Samber et al., *J. Anal. At. Spectrom.*, 2008, 23, 829-839.